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(54) Title: COMPOUNDS FOR DIAGNOSIS OF TUBERCULOSIS AND METHODS FOR THEIR USE

(57) Abstract

Compounds and methods for diagnosing tuberculosis are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of one or more *M. tuberculosis* proteins, and DNA sequences encoding such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of *M. tuberculosis* infection in patients and biological samples. Antibodies directed against such polypeptides are also provided.

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Description

COMPOUNDS FOR DIAGNOSIS OF TUBERCULOSIS AND METHODS FOR THEIR USE

Technical Field

The present invention relates generally to the detection of *Mycobacterium tuberculosis* infection. The invention is more particularly related to polypeptides comprising a *Mycobacterium tuberculosis* antigen, or a portion or other variant thereof, and the use of such polypeptides for the serodiagnosis of *Mycobacterium tuberculosis* infection.

Background of the Invention

Tuberculosis is a chronic, infectious disease, that is generally caused by infection with *Mycobacterium tuberculosis*. It is a major disease in developing countries, as well as an increasing problem in developed areas of the world, with about 8 million new cases and 3 million deaths each year. Although the infection may be asymptomatic for a considerable period of time, the disease is most commonly manifested as an acute inflammation of the lungs, resulting in fever and a nonproductive cough. If left untreated, serious complications and death typically result.

Although tuberculosis can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease. Infected individuals may be asymptomatic, but contagious, for some time. In addition, although compliance with the treatment regimen is critical, patient behavior is difficult to monitor. Some patients do not complete the course of treatment, which can lead to ineffective treatment and the development of drug resistance.

Inhibiting the spread of tuberculosis will require effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient method for inducing protective immunity. The most common Mycobacterium for this purpose is Bacillus Calmette-Guerin (BCG), an avirulent strain of *Mycobacterium bovis*.

However, the safety and efficacy of BCG is a source of controversy and some countries, such as the United States, do not vaccinate the general public. Diagnosis is commonly achieved using a skin test, which involves intradermal exposure to tuberculin PPD (protein-purified derivative). Antigen-specific T cell responses result in measurable incubation at the injection site by 48-72 hours after injection, which indicates exposure to Mycobacterial antigens. Sensitivity and specificity have, however, been a problem with this test, and individuals vaccinated with BCG cannot be distinguished from infected individuals.

While macrophages have been shown to act as the principal effectors of *M. tuberculosis* immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against *M. tuberculosis* infection is illustrated by the frequent occurrence of *M. tuberculosis* in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4 T cells have been shown to be potent producers of gamma-interferon (IFN-γ), which, in turn, has been shown to trigger the anti-mycobacterial effects of macrophages in mice. While the role of IFN-γ in humans is less clear, studies have shown that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-γ or tumor necrosis factor-alpha, activates human macrophages to inhibit *M. tuberculosis* infection. Furthermore, it is known that IFN-γ stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to *M. tuberculosis* infection. For a review of the immunology of *M. tuberculosis* infection *see* Chan and Kaufmann, in *Tuberculosis: Pathogenesis, Protection and Control*, Bloom (ed.), ASM Press, Washington, DC, 1994.

Accordingly, there is a need in the art for improved diagnostic methods for detecting tuberculosis. The present invention fulfills this need and further provides other related advantages.

Summary of the Invention

Briefly stated, the present invention provides compositions and methods for diagnosing tuberculosis.

In one embodiment, polypeptides are provided that comprise an antigenic portion of a *M. tuberculosis* antigen, or a variant of such an antigen that differs only in

conservative substitutions and/or modifications, wherein the antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited in SEQ ID NO: 1, 11, 12, 83, 103-108, 125, 127, 129-137, 139 and 140, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID NO: 1, 11, 12, 83, 103-108, 125, 127, 129-137, 139 and 140, or a complement thereof, under moderately stringent conditions. In a second embodiment, the present invention provides polypeptides comprising an immunogenic portion of a *M. tuberculosis* antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 16-33, 109, 126, 138, 141, 142 and variants thereof.

In related aspects, DNA sequences encoding the above polypeptides, recombinant expression vectors comprising these DNA sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known *M. tuberculosis* antigen.

In further aspects of the subject invention, methods and diagnostic kits are provided for detecting tuberculosis in a patient. The methods comprise: (a) contacting a biological sample with at least one of the above polypeptides; and (b) detecting in the sample the presence of antibodies that bind to the polypeptide or polypeptides, thereby detecting *M. tuberculosis* infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. The diagnostic kits comprise one or more of the above polypeptides in combination with a detection reagent.

The present invention also provides methods for detecting *M. tuberculosis* infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least one oligonucleotide primer in a polymerase chain reaction, the oligonucleotide primer being specific for a DNA sequence encoding the above polypeptides; and (c) detecting in the sample a DNA sequence that amplifies in the presence of the first and second oligonucleotide primers. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous nucleotides of such a DNA sequence.

In a further aspect, the present invention provides a method for detecting *M. tuberculosis* infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a DNA sequence encoding the above polypeptides; and (c) detecting in the sample a DNA sequence that hybridizes to the oligonucleotide probe. In one embodiment, the oligonucleotide probe comprises at least about 15 contiguous nucleotides of such a DNA sequence.

In yet another aspect, methods are provided for detecting tuberculosis in a patient, such methods comprising contacting a biological sample with one or more polypeptides encoded by a DNA sequence selected from the group consisting of SEQ ID NO: 2-10, 102, 128, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID NO: 2-10, 102, 128; and detecting in the sample the presence of antibodies that bind to the polypeptide, thereby detecting *M. tuberculosis* infection in the biological sample. Diagnostic kits for use in such methods are also provided.

In another aspect, the present invention provides antibodies, both polyclonal and monoclonal, that bind to the polypeptides described above, as well as methods for their use in the detection of *M. tuberculosis* infection.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Figures 1 and 1B illustrate the stimulation of proliferation and interferon-γ production, respectively, in T cells derived from a first PPD-positive donor (referred to as D7) by recombinant ORF-2 and synthetic peptides to ORF-2.

Figures 2A and 2B illustrate the stimulation of proliferation and interferon-γ production, respectively, in T cells derived from a second PPD-positive donor (referred to as D160) by recombinant ORF-2 and synthetic peptides to ORF-2.

Detailed Description of the Invention

As noted above, the present invention is generally directed to compositions and methods for preventing, treating and diagnosing tuberculosis. The compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a *M. tuberculosis* antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (*i.e.*, antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the above antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *M. tuberculosis* antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

"Immunogenic," as used herein, refers to the ability to elicit an immune response (*e.g.*, cellular) in a patient, such as a human, and/or in a biological sample. In particular, antigens that are immunogenic (and immunogenic portions or other variants of such antigens) are capable of stimulating cell proliferation, interleukin-12 production and/or interferon-γ production in biological samples comprising one or more cells selected from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from an *M. tuberculosis*-immune individual. Polypeptides comprising at least an immunogenic portion of one or more *M. tuberculosis* antigens may generally be used to detect tuberculosis or to induce protective immunity against tuberculosis in a patient.

The compositions and methods of this invention also encompass variants of the above polypeptides. A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such that the therapeutic, antigenic and/or immunogenic properties of the polypeptide are retained. Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity to the identified polypeptides. For polypeptides with immunoreactive properties, variants may, alternatively, be identified by modifying the amino acid sequence of one of the above polypeptides, and evaluating the immunoreactivity of the modified polypeptide. For polypeptides useful for the generation of

diagnostic binding agents, a variant may be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of tuberculosis. Alternatively, variants of the claimed antigens that may be usefully employed in the inventive diagnostic methods may be identified by evaluating modified polypeptides for their ability to detect antibodies present in the sera of tuberculosis-infected patients. Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.

A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

In general, *M. tuberculosis* antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, genomic or cDNA libraries derived from *M. tuberculosis* may be screened directly using peripheral blood mononuclear cells (PBMCs) or T cell lines or clones derived from one or more *M. tuberculosis*-immune individuals. Direct library screens may generally be performed by assaying pools of expressed recombinant proteins for the ability of induce proliferation and/or interferon-γ production in T cells derived from an *M. tuberculosis*-immune individual.

Potential T cell antigens may be first selected based on antibody reactivity, as described above.

Alternatively, DNA sequences encoding antigens may be identified by screening an appropriate *M. tuberculosis* genomic or cDNA expression library with sera obtained from patients infected with *M. tuberculosis*. Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989.

Purified antigens are then evaluated for their ability to elicit an appropriate immune response (e.g., cellular) using, for example, the representative methods described herein. Immunogenic antigens may then be partially sequenced using techniques such as traditional Edman chemistry. *See* Edman and Berg, *Eur. J. Biochem. 80*:116-132, 1967. Immunogenic antigens may also be produced recombinantly using a DNA sequence that encodes the antigen, which has been inserted into an expression vector and expressed in an appropriate host.

DNA sequences encoding the inventive antigens may also be obtained by screening an appropriate *M. tuberculosis* cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated antigens. Degenerate oligonucleotide sequences for use in such a screen may be designed and synthesized, and the screen may be performed, as described (for example) in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989 (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using the above oligonucleotides in methods well known in the art, to isolate a nucleic acid probe from a cDNA or genomic library. The library screen may then be performed using the isolated probe.

Regardless of the method of preparation, the antigens described herein are "antigenic." More specifically, the antigens have the ability to react with sera obtained from an *M. tuberculosis*-infected individual. Reactivity may be evaluated using, for example, the representative ELISA assays described herein, where an absorbance reading with sera from

infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals is considered positive.

Antigenic portions of *M. tuberculosis* antigens may be prepared and identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3d ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptide portions of the native antigen for antigenic properties. The representative ELISAs described herein may generally be employed in these screens. An antigenic portion of a polypeptide is a portion that, within such representative assays, generates a signal in such assays that is substantially similar to that generated by the full length antigen. In other words, an antigenic portion of a *M. tuberculosis* antigen generates at least about 20%, and preferably about 100%, of the signal induced by the full length antigen in a model ELISA as described herein.

Portions and other variants of *M. tuberculosis* antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc., Foster City, CA, and may be operated according to the manufacturer's instructions. Variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Sections of the DNA sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

Recombinant polypeptides containing portions and/or variants of a native antigen may be readily prepared from a DNA sequence encoding the polypeptide using a variety of techniques well known to those of ordinary skill in the art. For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an

affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides as described herein. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line, such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in substantially pure form. Preferably, the polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure. For use in the methods described herein, however, such substantially pure polypeptides may be combined.

In one embodiment, the subject invention discloses polypeptides comprising at least an antigenic portion of a *M. tuberculosis* antigen (or a variant of such an antigen) that comprises one or more of the amino acid sequences encoded by (a) the DNA sequences of SEQ ID NO: 1-12, 83, 102-108, 125, 127-137, 139 and 140, (b) the complements of such DNA sequences or (c) DNA sequences substantially homologous to a sequence in (a) or (b). In a related embodiment, the present invention provides polypeptides comprising at least an immunogenic portion of an *M. tuberculosis* antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 16-33, 109, 126, 138, 141, 142 and variants thereof.

The *M. tuberculosis* antigens provided herein include variants that are encoded by DNA sequences which are substantially homologous to one or more of DNA sequences specifically recited herein. "Substantial homology," as used herein, refers to DNA sequences that are capable of hybridizing under moderately stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species

homology, at 45°C with 0.5X SSC, followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS). Such hybridizing DNA sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode an immunogenic polypeptide that is encoded by a hybridizing DNA sequence.

In a related aspect, the present invention provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known *M. tuberculosis* antigen, such as the 38 kD antigen described in Andersen and Hansen, *Infect. Immun. 57*:2481-2488, 1989, (Genbank Accession No. M30046), or ESAT-6 previously identified in *M. bovis* (Accession No. U34848) and in *M. tuberculosis* (Sorensen et al., *Infect. Immun. 63*:1710-1717, 1995). Variants of such fusion proteins are also provided. The fusion proteins of the present invention may include a linker peptide between the first and second polypeptides.

A DNA sequence encoding a fusion protein of the present invention is constructed using known recombinant DNA techniques to assemble separate DNA sequences encoding the first and second polypeptides into an appropriate expression vector. The 3' end of a DNA sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids,

such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene 40*:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA 83*:8258-8562, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. Peptide linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric hindrance.

In another aspect, the present invention provides methods for using the polypeptides described above to diagnose tuberculosis. In this aspect, methods are provided for detecting *M. tuberculosis* infection in a biological sample, using one or more of the above polypeptides, alone or in combination. In embodiments in which multiple polypeptides are employed, polypeptides other than those specifically described herein, such as the 38 kD antigen described above, may be included. As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient or a blood supply. The polypeptide(s) are used in an assay, as described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative to a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to mycobacterial antigens which may be indicative of tuberculosis.

In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (*i.e.*, one component polypeptide will tend to detect infection in samples where the infection would not be detected by another component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with *M. tuberculosis*. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested. Such polypeptides are complementary. For example, approximately 25-30% of sera from tuberculosis-infected individuals are negative for antibodies to any single protein, such as the

38 kD antigen mentioned above. Complementary polypeptides may, therefore, be used in combination with the 38 kD antigen to improve sensitivity of a diagnostic test.

There are a variety of assay formats known to those of ordinary skill in the art for using one or more polypeptides to detect antibodies in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, which is incorporated herein by reference. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (e.g., in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The polypeptides may be bound to the solid support using a variety of techniques known to those of ordinary skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In

general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 µg, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (*see, e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO) may be employed. The immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is that period of time that is sufficient to detect the presence of antibody within a *M. tuberculosis*-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a

period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (*e.g.*, Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-*M. tuberculosis* antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one

preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for tuberculosis. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, pp. 106-107. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for tuberculosis.

In a related embodiment, the assay is performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (*e.g.*, protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-*M. tuberculosis* antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the

biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 μg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (*e.g.*, one drop) of patient serum or blood.

Of course, numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only.

In yet another aspect, the present invention provides antibodies to the inventive polypeptides. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the antigenic polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep and goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be

employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Antibodies may be used in diagnostic tests to detect the presence of *M. tuberculosis* antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting *M. tuberculosis* infection in a patient.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify *M. tuberculosis*-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

As used herein, the term "oligonucleotide primer/probe specific for a DNA molecule" means an oligonucleotide sequence that has at least about 80%, preferably at least about 90% and more preferably at least about 95%, identity to the DNA molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (see, for example, Mullis *et al. Ibid*; Ehrlich, *Ibid*). Primers or probes may thus be used to detect *M. tuberculosis*-specific sequences in biological samples. DNA probes or primers comprising oligonucleotide sequences described above may be used alone, in combination with each other, or with previously identified sequences, such as the 38 kD antigen discussed above.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1

PURIFICATION AND CHARACTERIZATION OF *M. TUBERCULOSIS* POLYPEPTIDES USING CD4+ T CELL LINES GENERATED FROM HUMAN PBMC

M. tuberculosis antigens of the present invention were isolated by expression cloning of cDNA libraries of *M. tuberculosis* strains H37Rv and Erdman essentially as described by Sanderson et al. (*J. Exp. Med.*, 1995, *182*:1751-1757) and were shown to induce PBMC proliferation and IFN-γ in an immunoreactive T cell line.

Two CD4+ T cell lines, referred to as DC-4 and DC-5, were generated against dendritic cells infected with *M. tuberculosis*. Specifically, dendritic cells were prepared from adherent PBMC from a single donor and subsequently infected with tuberculosis. Lymphocytes from the same donor were cultured under limiting dilution conditions with the infected dendritic cells to generate the CD4+ T cell lines DC-4 and DC-5. These cell lines were shown to react with crude soluble proteins from *M. tuberculosis* but not with Tb38-1. Limiting dilution conditions were employed to obtain a third CD4+ T cell line, referred to as DC-6, which was shown to react with both crude soluble proteins and Tb38-1.

Genomic DNA was isolated from the *M. tuberculosis* strains H37Rv and Erdman and used to construct expression libraries in the vector pBSK(-)using the Lambda ZAP expression system (Stratagene, La Jolla, CA). These libraries were transformed into *E. coli*, pools of induced *E. coli* cultures were incubated with dendritic cells, and the ability of the resulting incubated dendritic cells to stimulate cell proliferation and IFN-γ production in the CD4+ T cell line DC-6 was examined as described below in Example 2. Positive pools were fractionated and re-tested until pure *M. tuberculosis* clones were obtained.

Nineteen clones were isolated, of which nine were found to contain the previously identified *M. tuberculosis* antigens TbH-9 and Tb38-1, disclosed in U.S. Patent Application Nos. 08/533,634. The determined cDNA sequences for the remaining ten clones (hereinafter referred to as Tb224, Tb636, Tb424, Tb436, Tb398, Tb508, Tb441, Tb475, Tb488 and Tb465) are provided in SEQ ID No: 1-10, respectively. The corresponding predicted amino acid sequences for Tb224 and Tb636 are provided in SEQ ID NO: 13 and 14, respectively. The open reading frames for these two antigens were found to show some homology to TbH-9. Tb224 and Tb636 were also found to be overlapping clones.

Tb424, Tb436, Tb398, Tb508, Tb441, Tb475, Tb488 and Tb465 were each found to contain two small open reading frames (referred to as ORF-1 and ORF-2) or truncated forms thereof, with minor variations in ORF-1 and ORF-2 being found for each clone. The predicted amino acid sequences of ORF-1 and ORF-2 for Tb424, Tb436, Tb398, Tb508, Tb441, Tb475, Tb488 and Tb465 are provided in SEQ ID NO: 16 and 17, 18 and 19, 20 and 21, 22 and 23, 24 and 25, 26 and 27, 28 and 29, and 30 and 31, respectively. In addition, clones Tb424 and Tb436 were found to contain a third apparent open reading frame, referred to as ORF-U. The predicted amino acid sequences of ORF-U for Tb424 and Tb436 are provided in SEQ ID NO: 32 and 33, respectively. Tb424 and Tb436 were found to be either overlapping clones or recently duplicated/transposed copies. Similarly Tb398, Tb508 and Tb465 were found to be either overlapping clones or recently duplicated/transposed copies, as were Tb475 and Tb488.

These sequences were compared with known sequences in the gene bank using the BLASTN system. No homologies to the antigens Tb224 and Tb431 were found. Tb636 was found to be 100% identical to a cosmid previously identified in *M. tuberculosis*. Similarly, Tb508, Tb488, Tb398, Tb424, Tb436, Tb441, Tb465 and Tb475 were found to show homology to known *M. tuberculosis* cosmids. In addition, Tb488 was found to have 100% homology to *M. tuberculosis* topoisomerase I.

Seventeen overlapping peptides to the open reading frames ORF-1 (referred to as 1-1 - 1-17; SEQ ID NO: 34-50, respectively) and thirty overlapping peptides to the open reading frame ORF-2 (referred to as 2-1 - 2-30, SEQ ID NO: 51-80) were synthesized using the procedure described below in Example 3.

The ability of the synthetic peptides, and of recombinant ORF-1 and ORF-2, to induce T cell proliferation and IFN-γ production in PBMC from PPD-positive donors was assayed as described below in Example 2. Figs. 1A-B and 2A-B illustrate stimulation of T cell proliferation and IFN-γ by recombinant ORF-2 and the synthetic peptides 2-1 - 2-16 for two donors, referred to as D7 and D160, respectively. Recombinant ORF-2 (referred to as MTI) stimulated T cell proliferation and IFN-γ production in PBMC from both donors. The amount of PBMC stimulation seen with the individual synthetic peptides varied with each donor, indicating that each donor recognizes different epitopes on ORF2. The proteins encoded by ORF-1, ORF-2 and ORF-U were subsequently named MTS, MTI and MSF, respectively.

Eighteen overlapping peptides to the sequence of MSF (referred to as MSF-1 - MSF-18; SEQ ID NO: 84-101, respectively) were synthesized and their ability to stimulate T cell proliferation and IFN-γ production in a CD4+ T cell line generated against *M. tuberculosis* culture filtrate was examined as described below. The peptides referred to as MSF-12 and MSF-13 (SEQ ID NO: 95 and 96, respectively) were found to show the highest levels of reactivity.

Two overlapping peptides (SEQ ID NO:81 and 82) to the open reading frame of Tb224 were synthesized and shown to induce T cell proliferation and IFN-γ production in PBMC from PPD-positive donors.

Two CD4+ T cell lines from different donors were generated against *M. tuberculosis* infected dendritic cells using the above methodology. Screening of the *M. tuberculosis* cDNA expression library described above using this cell line, resulted in the isolation of two clones referred to as Tb867 and Tb391. The determined cDNA sequence for Tb867 (SEQ ID NO: 102) was found to be identical to the previously isolated *M. tuberculosis* cosmid SCY22G10, with the candidate reactive open reading frame encoding a 750 amino acid *M. tuberculosis* protein kinase. Comparison of the determined cDNA sequence for Tb391 (SEQ ID NO: 103) with those in the gene bank revealed no significant homologies to known sequences.

In further studies, CD4+ T cell lines were generated against *M. tuberculosis* culture filtrate, essentially as outlined above, and used to screen the *M. tuberculosis* Erdman cDNA expression library described above. Five reactive clones, referred to as Tb431, Tb472, Tb470, Tb838 and Tb962 were isolated. The determined cDNA sequences for Tb431, Tb472, Tb470, and Tb838 are provided in SEQ ID NO: 11, 12, 104 and 105, respectively, with the determined cDNA sequences for Tb962 being provided in SEQ ID NO: 106 and 107. The corresponding predicted amino acid sequence for Tb431 is provided in SEQ ID NO: 15.

Subsequent studies led to the isolation of a full-length cDNA sequence for Tb472 (SEQ ID NO: 108). Overlapping peptides were synthesized and used to identify the reactive open reading frame. The predicted amino acid sequence for the protein encoded by Tb472 (referred to as MSL) is provided in SEQ ID NO: 109. Comparison of the sequences for Tb472 and MSL with those in the gene bank, as described above, revealed no homologies to known sequences. Fifteen overlapping peptides to the sequence of MSL (referred to as MSL-1 – MSL-15; SEQ ID NO: 110-124, respectively) were synthesized and their ability to stimulate T cell proliferation and IFN-γ production in a CD4+ T cell line generated against *M. tuberculosis* culture filtrate was examined as described below. The peptides referred to as MSL-10 (SEQ ID NO: 119) and MSL-11 (SEQ ID NO: 120) were found to show the highest level of reactivity.

Comparison of the determined cDNA sequence for Tb838 with those in the gene bank revealed identity to the previously isolated *M. tuberculosis* cosmid SCY07H7. Comparison of the determined cDNA sequences for the clone Tb962 with those in the gene

bank revealed some homology to two previously identified *M. tuberculosis* cosmids, one encoding a portion of bactoferritin. However, recombinant bactoferritin was not found to be reactive with the T cell line used to isolate Tb962.

The clone Tb470, described above, was used to recover a full-length open reading (SEQ ID NO: 125) that showed homology with TbH9 and was found to encode a 40 kDa antigen, referred to as Mtb40. The determined amino acid sequence for Mtb40 is provided in SEQ ID NO: 126. Similarly, subsequent studies led to the isolation of the full-length cDNA sequence for Tb431, provided in SEQ ID NO: 83, which was also determined to contain an open reading frame encoding Mtb40. Tb470 and Tb431 were also found to contain a potential open reading frame encoding a U-ORF-like antigen.

Screening of an *M. tuberculosis* Erdman cDNA expression library with multiple CD4+ T cell lines generated against *M. tuberculosis* culture filtrate, resulted in the isolation of three clones, referred to as Tb366, Tb433 and Tb439. The determined cDNA sequences for Tb366, Tb433 and Tb439 are provided in SEQ ID NO: 127, 128 and 129, respectively. Comparison of these sequences with those in the gene bank revealed no significant homologies to Tb366. Tb433 was found to show some homology to the previously identified *M. tuberculosis* antigen MPT83. Tb439 was found to show 100% identity to the previously isolated *M. tuberculosis* cosmid SCY02B10.

A CD4+ T cell line was generated against *M. tuberculosis* PPD, essentially described above, and used to screen the above *M. tuberculosis* Erdman cDNA expression library. One reactive clone (referred to as Tb372) was isolated, with the determined cDNA sequences being provided in SEQ ID NO: 130 and 131. Comparison of these sequences with those in the gene bank revealed no significant homologies.

In further studies, screening of an *M. tuberculosis* cDNA expression library with a CD4+ T cell line generated against dendritic cells that had been infected with tuberculosis for 8 days, as described above, led to the isolation of two clones referred to as Tb390R5C6 and Tb390R2C11. The determined cDNA sequence for Tb390R5C6 is provided in SEQ ID NO: 132, with the determined cDNA sequences for Tb390R2C11 being provided in SEQ ID NO: 133 and 134. Tb390R5C6 was found to show 100% identity to a previously identified *M. tuberculosis* cosmid.

In subsequent studies, the methodology described above was used to screen an *M. tuberculosis* genomic DNA library prepared as follows. Genomic DNA from *M tuberculosis* Erdman strain was randomly sheared to an average size of 2 kb, and blunt ended with Klenow polymerase, followed by the addition of EcoRI adaptors. The insert was subsequently ligated into the Screen phage vector (Novagen, Madison, WI) and packaged *in vitro* using the PhageMaker extract (Novagen). The phage library (referred to as the Erd λ Screen library) was amplified and a portion was converted into a plasmid expression library by an autosubcloning mechanism using the *E. coli* strain BM25.8 (Novagen). Plasmid DNA was purified from BM25.8 cultures containing the pSCREEN recombinants and used to transform competent cells of the expressing host strain BL21(DE3)pLysS. Transformed cells were aliquoted into 96 well microtiter plates with each well containing a pool size of approximately 50 colonies. Replica plates of the 96 well plasmid library format were induced with IPTG to allow recombinant protein expression. Following induction, the plates were centrifuged to pellet the *E. coli* which was used directly in T cell expression cloning of a CD4+ T cell line prepared from a PPD-positive donor (donor 160) as described above. Pools containing *E. coli* expressing *M. tuberculosis* T cell antigens were subsequently broken down into individual colonies and reassayed in a similar fashion to identify positive hits.

Screening of the T cell line from donor 160 with one 96 well plate of the Erd λ Screen library provided a total of nine positive hits. Previous experiments on the screening of the pBSK library described above with T cells from donor 160 suggested that most or all of the positive clones would be TbH-9, Tb38-1 or MTI (disclosed in U.S. Patent Application No. 08/533,634) or variants thereof. However, Southern analysis revealed that only three wells hybridized with a mixed probe of TbH-9, Tb38-1 and MTI. Of the remaining six positive wells, two were found to be identical. The determined 5' cDNA sequences for two of the isolated clones (referred to as Y1-26C1 and Y1-86C11) are provided in SEQ ID NO: 135 and 136, respectively. The full length cDNA sequence for the isolated clone referred to as hTec#1 is provided in SEQ ID NO: 137, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 138. Comparison of the sequences of hTec#1 to those in the gene bank as described above, revealed some homology to the previously isolated *M. tuberculosis* cosmid MTCY07H7B.06

EXAMPLE 2

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-γ PRODUCTION BY *M. TUBERCULOSIS* ANTIGENS

The ability of recombinant *M. tuberculosis* antigens to induce T cell proliferation and interferon-γ production may be determined as follows.

Proteins may be induced by IPTG and purified by gel elution, as described in Skeiky et al. *J. Exp. Med.*, 1995, *181*:1527-1537. The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. The PBMCs from donors known to be PPD skin test positive and whose T-cells are known to proliferate in response to PPD, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μg/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 μg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μl, 50 μl of medium is removed from each well for determination of IFN-γ levels, as described below. The plates are then pulsed with 1 μCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ is measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate

added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

EXAMPLE 3

PURIFICATION AND CHARACTERIZATION OF *M. TUBERCULOSIS* POLYPEPTIDES USING CD4+ T CELL LINES GENERATED FROM A MORISE *M. TUBERCULOSIS* MODEL

Infection of C57BL/6 mice with *M. tuberculosis* results in the development of a progressive disease for approximately 2-3 weeks. The disease progression is then halted as a consequence of the emergence of a strong protective T cell-mediated immune response. This infection model was used to generate T cell lines capable of recognizing protective *M. tuberculosis* antigens.

Specifically, spleen cells were obtained from C57BL/6 mice infected with *M tuberculosis* for 28 days and used to raise specific anti-*M. tuberculosis* T cell lines as described above. The resulting CD4+ T cell lines, in conjunction with normal antigen presenting (spleen) cells from C57BL/6 mice were used to screen the *M. tuberculosis* Erd λ screen library described above. One of the reactive library pools, which was found to be highly stimulatory of the T cells, was selected and the corresponding active clone (referred to as Y288C10) was isolated.

Sequencing of the clone Y288C10 revealed that it contains two potential genes, in tandem. The determined cDNA sequences for these two genes (referred to as mTCC#1 and mTCC#2) are provided in SEQ ID NO: 139 and 140, respectively, with the corresponding predicted amino acid sequences being provided in SEQ ID NO: 141 and 142, respectively. Comparison of these sequences with those in the gene bank revealed identity to unknown sequences previously found within the *M. tuberculosis* cosmid MTY21C12. The predicted amino acid sequences of mTCC#1 and mTCC#2 were found to show some homology to previously identified members of the Tb19 protein family, discussed above.

EXAMPLE 4

SYNTHESIS OF SYNTHETIC POLYPEPTIDES

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray mass spectrometry and by amino acid analysis.

EXAMPLE 4

USE OF REPRESENTATIVE ANTIGENS FOR SERODIAGNOSIS OF TUBERCULOSIS

The diagnostic properties of representative *M. tuberculosis* antigens may be determined by examining the reactivity of antigens with sera from tuberculosis-infected patients and from normal donors as described below.

Assays are performed in 96-well plates coated with 200 ng antigen diluted to 50 µL in carbonate coating buffer, pH 9.6. The wells are coated overnight at 4°C (or 2 hours at 37°C). The plate contents are then removed and the wells are blocked for 2 hours with 200 µL of PBS/1% BSA. After the blocking step, the wells are washed five times with PBS/0.1% Tween 20™. 50 µL sera, diluted 1:100 in PBS/0.1% Tween 20™/0.1% BSA, is

then added to each well and incubated for 30 minutes at room temperature. The plates are washed again five times with PBS/0.1% Tween 20™.

The enzyme conjugate (horseradish peroxidase - Protein A, Zymed, San Francisco, CA) is then 1:10,000 in PBS/0.1% Tween 20™/0.1% BSA, and 50 µL of the diluted conjugate is added to each well and incubated for 30 minutes at room temperature. Following incubation, the wells are washed five times with PBS/0.1% Tween 20™. 100 µL of tetramethylbenzidine peroxidase (TMB) substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) is added, undiluted, and incubated for about 15 minutes. The reaction is stopped with the addition of 100 µL of 1 N H_2SO_4 to each well, and the plates are read at 450 nm.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: COMPOUNDS AND DIAGNOSIS OF TUBERCULOSIS AND METHODS OF THEIR USE

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Patentin Release #1.0, Version #1.30

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1886 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CGCTCTGGTG ACCACCAACT TCTTCGGTGT CAACACCGTC CCGATCGCCC TCAACGAGGC      60
CGACTACCTG CGCATGTGGA TCTACGGCCG CACCGTCATG AGCCACTATC AACCCGTCGC     120
GCACGAAATC TGGTCTCTCG ATGAATAAGG CAGTTCCGGA AAGCGCTGGG CCAGTATCAC     180
CACGGGTGCG CCGGGCTCAC CGGCCTCGAC CACTCGCAGT CGCACGCCGT TGGTATCAAC     240
TRACCGTNCN GTANGTGCGC CCATGGTCTC ACGAAATCAC ACCGGGCAGC GGCCTGAGAA     300
GGGCTTGGGG AGCAACCAGA GGCGATTGTC GCGGTTGCTG CCGCGCATCA TTGATCGGCC     360
GGCCGGACCA NTCGGGCCTC CCTTGACGTC CGCATCNCAC TTCCTGTGCA GCTGGCATGG     420
CTACAGCTCA CAGTGACTGC CCCACGATTG CCGGCCAGGT CGAGTTCAAA TTCCGGTTGA     480
TTCGCGGACA AAAGCAGCAG GTCAACCAAC CGCAGTCAGT CTAGGGTCCC AAACTTGAGC     540
CAATCGGTGA AATGGCTTGC TGCAGTGACA CCGGTCACAG GCTTAGCCGA CATCACCGGA     600
ATAGCTCAGG CGGCCTATAG AGTCCTATAG AAACATTTGC TGATAGAATT AACCGCTGTC     660
TTGGCGTGAT CTTGATACGG CTTCGCCGTG CGACCGGTTG CTCAGTAGCT GACCACCATG     720
TAACCCATTC TCGGCAGGTG TCTACTAAGG CGAGACACCG CATTGGTGGC GCTGCATCGC     780
AAATCGGTCC GAGCATGTAG CACTGCTGTT ATCCCCGGAT AGCAAACCAC CCGGAACCAG     840
GGCTATCCCA GTCGCTCCTC GACGGAGGCC GTTTCGCTTT CCGTTGCCCG ATAACTCCCG     900
AGTGGATATC GCCGTTATCA NATTCAGGCT TTTCTTCGCA AGTTACCGGT GTTCGCTATA     960
TTCGGATATC TCCGACGGAT AATTACTAAA ACTTCAGTGG TTTAGATAAG GCCGCCGCAA    1020
TACTTCGCCG ATCTTGCCGA GCGCAACGGA TTTCGATCGT CGTTTTCTGT CGCCTTATCA    1080
AACATGATCG GAGATAATGA CAGATCGGCC TAGCTAGTTG TTTAGCGTAC GCGATTTAGG    1140
ACAACCGAGA TTTGTTTTGC CTCGCAACCA TGAGAGCGCC CCGCTTCGAC GCCGAATCGG    1200
GTNANTGANG GTNGGGTTAG CACGCCCTGA TTGCGCCACC GGCGAGGTGA TTTTCCCCGC    1260
CACGAAGCCG CCGCCGGCTA GCCCCATGAG CACGTTATAT AGACTTCTCT GCAACAGATC    1320
TCATACCGAT CGAAGCGAAA GCGCAGCAT CGACGTCGGA GACACTGCCT TGGGATCGCG    1380
CCGCCTACAC GGCGGTTCGC GCATTNTCGC AGCCACGTTG CAGGAGGCCA AATGTGCGCA    1440
GACGATGTAG TCGACAACAA GTGNACATGC CGTCTTCACG AACTCAAAAC TGACGATCTG    1500
CTTAGCATGA AAAAAACTGT TGACATCGGC CAAGCATGAC AGCCAGACTG TAGGCCTACG    1560
CGTGCAATGC AGAACCAAGG NTATGCATGG AATCGACGAC CGTTGAGATA GGCGCCAGGC    1620
ATGAGCGGAG CGTTCATCAT CGATCCAACG ATCAGTGCCA TCGACGGCTT GTACGACCTT    1680
CTGGGGATTG GAATACCCAA CCAAGGGGTT AICCTTTACT CCTCACTAGA GTACTTCGAA    1740
AAAGCCCTGG AGGAGCTGGC AGCAGCGTTT CCGGCTGATG GCTTCTTAGG TTCGCTGCGC    1800
GACAAATACG CCGGCAAAAA CCGCAACCAC GTGAATTTTT TCCAGGAACT GGCAGACCTC    1860
GATCGTCAGC TCATCAGCCT GATCCA                                         1886
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2305 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GGCACGCGCT GGCCGCGCAA TACACCGAAA TTGCAACGGA ACTCGCAAGC GTGCTCGCTG      60
CGGTGCAGGC AAGCTCGTGG CAGGGGCCCA GCGCCGACCG GTTCGTCGTC GCCCATCAAC     120
CGTTCCGGTA TTGGCTAACC CACGCTGCCA CGTTGGCCAC CGCAGCAGCC GCCGCGCATN     180
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AAACGGCCGC CGCCGGGTAT ACGTCCGCAT TGGGGGGCAT GCCTACGCTA GCCGACTTGG      240
CGGCCAACCA TGCCATGCAC GGCGCTCTCTGG TGACCACCAA CTTCTTCGTT GTCAACACCA      300
TCCCGATCGC CCTCAACGAG GCCGACTACC TCCGCATTTG GATCCAGGCC GCCACCGTCA      360
TGAGCCACTA TCAAGCCGTC GCGCACGAAA GCGTGGCGGC GATCCCCAGC ACGCCGCGGC      420
CGCCGCAGAT AGTGACCAGT GCGGCCAGCT CGGCGGTTAG CAGCAGCTTC CCCGACCCGA      480
CCAAATTGAT GCTGCAGCTA CTCAAGGATT TCCTTGAGCT GCTGCGCTAT CTGGCTGTTG      540
AGCTGCTGCC GGGCCCGCTC GCCGACTTCA TCGCCCAGGT GTTGGACTGG TTCATCTCGT      600
TCGTTGTCGG TCCAGTCTTC ACGTTTCTCG CGTACCTTGT GCTGGACCCA CTGATCTATT      660
TCGGACCGTT CGCCGCGCTG ATGAGTCCGG TCCTGTTTGC TGCTGTGGAG TTACGCAACC      720
GCCTCAAAAC CGCCACCGGA CTGACGCTGC CACCTACCGT GATTTTCGAT CATCCCACTC      780
CCACTGCGGT CGCCGAGTAT GTCGCCACGC AAATGTCTGG CAGCCGCCCA ACGGAATCCG      840
GTGATCCGAC GTCGCAGGTT GTCGAACCCG CTCGTGCCGA ATTCGGCACG AGTGCTGTTC      900
ATCAAATCCC CCCGAGACCT GCGGACACCC GGCGCGCTTG CTGACATCGA GATGATGTCC      960
CGCGAGATAG CAGAATTGCC CAACATTGTG ATGGTGCGGG GCTTGACCCG ACCGAACGGC     1020
GAACTCTTGA AGGAGACCAA GGTCTCGTTT CAGGCTGGTG AAGTGGGCGG CTAGCTCGAC     1080
GAAGCGACCA CCTGCTCGA AGAGCACGGA GGCGAGCTGG ACCACGTGAC CGGCGGTGCG     1140
CACCAGTTGG CGGACGCCGT CGCCCAAATA CGCAADGAAA TCAATGGGGC CGTGGCCAGC     1200
TGGAGCGGGA TAGTCAACAC CGTGCAGGCC ATGATGAACC TGATGGGGGG TGACAAGACC     1260
ATCCGACAAC TGGAAAATGC GTCCGAATAT CTCGGGCCCA TGCCAGCCTT GGGGGACAAT     1320
CTGAGCGGGA CCGTCACCGA TGCCGAACAA ATCGCCACTT GGGCCAGCCC TATGGTCAAC     1380
GCCGTCAACT CCAGCCCGGT GTGTAACAGC GATCCCGCCT GTGGGACGTC GCCGCGACAG     1440
TTGGCGGCGA TTGTCCAGGC GCAGGACGAC GGCCTGCTCA GGTCCATCAG AGCGCTAGCC     1500
GTCACCGTGC AACAGACGCA GGATACCAG ACACTCGCCC GGACGGTGAG CACACTGGAC     1560
GGCCAACTGA AGCAAGTCGT CAGCACCCTC AAAGCGGTCG ACGGCCTACC CACCAAATTG     1620
GCTCAAATGC AGCAAGGAGC CAACGCTCTC GCCGACGGCA GTGCAGCGCT GGCGGCAGGC     1680
GTGCAGGAAT TGGTCGATCA GGTCAAAAAG ATGGGCTCAG GGCTCAACGA GGCCGCCGAC     1740
TTCCTGTTGG GGATCAAGCG GGATGCGGAC AAGCCGTCAA TGGCGGGCTT CAACATTCCA     1800
CCGCAGATTT TTCGAGGGA CGAGTCCAAG AAGGGCGCCC AGATTTTCCT GTCGGCCGAT     1860
GGTCATGCGG CGCGGTACTT CGTGCAGAGC GCGCTGAATC CGGCCACCAC CAAGGCGATG     1920
GATCAGGTCA ACGATATCCT CGGTGTTGCG GATTCCGGGC GACCGAATAC CGAACTCGAG     1980
GATGCCACGA TAGTTCTGGC GGUGGTTCCG ACTGCGCTGC GGGATATCCG CGACTACTAC     2040
AACAGCGATA TGAAATTCAT CGTCATTGCG ACGATCGTTA TCGTATTCTT GATTTCTCTC     2100
ATTCTGNTGG GCGCACTTGT GNNTCCGATA TATCTGATAG GCTCGGTGCT GATTTCTTAC     2160
TTGTCGGCCG TAGGCATAGG AACTTTCGTT TTCCAATTGA TACTGGGCCA GGAAATGCAT     2220
TGGAGCCTGC CGGGACTGTC CTTCATATTA TTGGTTGCCA TCGGCGCTGA CTACAACATC     2280
CTGCTCATTT CACGCATCCG CGACG                                           2305

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1742 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Mycobacterium tuberculosis

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCGCTCTCTT TCAACGTCAT AAGTTCGGTG GGTCAGTCGG CCGCGCGTGC ATATGGCACC       60
AATAACGCGT GTCCCATTGA TACCCGAACC GCACGACGGT AGAGCGGATC AGCGCAGCCG      120
GTGCCGAACA CTACCGCGTC CACGCTCAGC CCTGCTGCGT TCCGGAAGAT CGAGCCTAGG      180
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TTCTCATTGT CGTTAACGCC TTCCAACACT GCGACGGTGC GCGCCCCGGC GACCACCTGA 240
GCAACGCTCG GCTCCGGCAC CCGGCGCGCC GCTCGCAACA CCCCACGATT GAGATCGAAG 300
CCGATCACCG GTGCCATGAC ATCAGCTGAG GCTCGATATT ACCGCCGCCC GACACCGGCC 360
AGATCATCCT TGAGCTTCGC CAGCCGGCGG TCGGTGCCGA ACAGCCGCAG CGGCGTGAAC 420
CGTCAGCACC GCATGCCTGG CACCACCAGC ACACCCTCGG CGATCAUCAA CGTCCTTCGG 480
GTCGGCACAT CGGGANNAGN GTCGATGCTG TTCAGGTCAC GGAAATCGTC GAGCCGTGGG 540
TCGTCGGGAT CGCNGACGTC GTAACATCG AGCCGTCGG GTTGCTGGC ACAACGCCTT 600
TCGTTCANGG GCTTTCGTCG ACCAGAGCCA GCATCAGATC GGCGGCGCTG CGCAGGATGT 660
CACGCTCGCT GCGGTTCAGC GTCGCGAGCC GCTCAGTCAG CCACTCTTGC AGAGAGCCGT 720
TGCTGGGATT AATTGGGAGA GGAAGACAGC ATGTCGTTCG TGACCACACA GCCGGAAGCC 780
CTGGCAGCTG CGGCGGCGAA CCTACAGGGT ATTGGCACGA CAATGAACGC CCAGAACGCG 840
GCCGCGGCTG CTGCAACCAC CGGAGTAGTG CCCGCAGCCG CGGATGAAGT ATCAGCGCTG 900
ACCGCGGCCC AGTTTGCTGC GCACGCGCAG ATGTACCAAA CGGTCAGCGC CCAGGCCGCG 960
GCCATTCACG AAATGTTCGT GAACACGCTG GTGGCCAGTT CTTGCTCATA CGCGGCCACC 1020
GAGGCGGCCA ACGCAGCCGC TGCCGGCTGA ACGGGCTCGC ACGAACCTGC TGAAGGAGAG 1080
GGGGAACGTT CGGAGTTCTC GGGTCAGGGG TTCGCCAGC GCCCAGCGGA TTCAGTATC 1140
GGCGTCCATA ACGACGAACG ATCTAGGCAT TCAGTACTAA GGAGACAGGC AACATGGCCT 1200
CACGTTTTAT GACGGATCCG CATGCGATGC GGGACATGGC GGCCCGTTTT GAGGTGCACG 1260
CCCAGACCGT CGAGGACGAG GCTCGCCGGA TGTGGGCGTC CGCGCAAAAC ATTTCCGGTG 1320
CGGGCTCGAG TGGCATGGCG GAGGCGACCT CGGTAGACAC CATGACCTAG ATGAATCAGG 1380
CGTTTCGCCA CATCGTGAAC ATCGTCGCAG GGGTGGCTGA CGGGCTGGTT CGCGACGCCA 1440
ACAATACGAA ACAGCAAGAG CAGGCCTCCC AGCAGATCCT GAGCAGNTAG CGCCGAAAGC 1500
CACAGCTGNG TACGTTTTCT CACATTACGA GACACAGAAT ATGACGATTA ATTATCAGTT 1560
CGGGGACGTC GACGCTCATG GCGCCGTGAT CCGCGCTCAG GCGGCGTCGC TTGAUGCGGA 1620
GCATCAGCCC ATCGTTCGTG ATGTGTTGGC CGCCGGTGAC TTTTGGGGCG GCGCCGGTTC 1680
GGTGGCTTGC CAGAAGTTCA TTACCCAGTT GGGCGGTAAC TTCGAGGTGA TCTACGAGCA 1740
GG 1742

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2836 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTTGATTCCG TTCGCGGCGC CGCCGAAGAC CACCAACTCC GCTGGGGTGG TCGCACAGGC 60
GTTTGCGTCG GTCAGCTGGC CGAATTCCAA TGATTGGTGG CTGGTTGCGG TTGCTGGCCT 120
CGATTACCCC CACGGAAACG ACGACGATCG TTCGTTTGCT CGGTCAGTCG TACTTCGGGA 180
CGGGCATGGC GCAGTTTCTT ACCTCGATCG CACAGCAGCT GAACTTCCGC CCAGGGGCCA 240
CAACGCTCGG CTCCGGCGGA GCCTGGTACC CAACGCCACA ATTCGCCGGC CTGGGTGCAG 300
GCCTGCCCGT GTCGGAGGAT TTGGCGCGGG CGGAACGCGT CGGGAAGTTG TCGGTGCCGC 360
CAAGTTGGGC CGTCGCGGCT CCGGCCTTCG CGGAGAAGCC TGAGCGGGGC ACGCCGATGT 420
CCGTCACCGG CGAAGCGTCC AGCTCCGGTC AGGGAGGCCT GCTTCGAGGC ATACCGCTGC 480
CGAGACGGGG GCGCCGTACA GGCGCCTTCG CTCACCGATA CGCGTTCCGC CACAGCGTGA 540
TTACCCGTCG TCCGTTCGCC GGATAGCTTT CGATCCGGTC TGCGCGGCCG CCGGAAATAC 600
TGCAGATAGC GATCGACCGC GCCGGTCGGT AAACGCCGCA CACGGCACTA TCAATGCGCA 660
CGGCGGGCGT TGATGCCAAA TTGACCGTCC CGACGGGGCT TTATCTGCGG CAAGATTTCA 720

TCCCCAGCCC GGTCGGTTGG CCGATAAATA CGCTGGTCAG CGCGACTCTT CCGGCTGAAT 780
TCGATTCTCT CGGCCGCCGC TCGACGCCGA GTATCTCGAG TGGCCGCGAA ACCCGGTCAA 840
ACGCTGTTAC TGTGGCGTTA CCACAGGTGA ATTTGCGGTG CCAACTGGTG AACACTTGCC 900
AACGGGTGGC ATCGAAATCA ACTTGTTGCG TTGCAGTGAT CTACTCTCTT GCAGAGAGCC 960
GTTGCTTGGA TTAATTGGGA GAGGAAGACA GCATGTCGTT CGTGACCACA CAGCCGAAAG 1020
CCCTGGCAGC TGCGGCGGCG AACCTACAGG GTATTGGCAC GACAATGAAC GCCGAGAACG 1080
CGGCCGGGTC TGCTCCAACC ACCGGAGTAG TGCCCGCAGC CGCCGATGAA GTATCAGCGC 1140
TGACCGCGGC TCAGTTTGCT GCGCACGCGC AGATGTACCA AACGGTCAGC GCCCAGGCCG 1200
CGGCCATTCA CGAAATGTTC GTGAACACGC TGGTGGCCAG TTCTGGCTCA TACGCGGCCA 1260
CCGAGGCCGC CAACGCAGCC GCTGCCGGCT GAACGGCTCC GCACGACCTT GCTGAAGGAG 1320
AGCGGGAACA TCCGGAGTTC TCGGGTCAGG GGTTGCGCCA GCGCCCAGCC GATTCAGCTA 1380
TCGGCGTCCA TAACAGCAGA CGATCTAGGC ATTCAGTACT AAGGAGACAG GCAACATGGC 1440
CTCACGTTTT ATGACGGATC CGCATGCGAT GCGGGACATG GCGGCCGGTT TTGAGGTGCA 1500
CGCCCAGACG GTGGAGGACG AGGCTCGCCG GATGTGGGCG TCCGCGCAAA ACATTTCCGG 1560
TCCGGGCTGG AGTGGCATGG CCGAGGCGAC CTCGCTAGAC ACCATGACCT AGATGAATCA 1620
GGCGTTTCGC AACATCGTGA ACATGCTGCA CGGGGTGGT GACGGCGTGC TTCGCGACGC 1680
CAACAACTAC GAACAGCAAG ACCAGCCCTC CCAGCAGATC CTGAGCAGCT AGCGCCGAAA 1740
GCCACAGCTG CGTAGCGTTT CTCACATTAG GAGAACACCA ATATGACGAT TAATTACCAG 1800
TTCGTGGACG TCGACGCTCA TGGCGCCATG ATCCGCGCTC AGGCGGCGTC GCTTGAGGCG 1860
GAGCATCAGG CCATCGTTCG TGATGTGTTG GCCGCCGGTG ACTTTTGGGG CGGCGCCGGT 1920
TCGGTGACTT GCCAGGAGTT CATTACTCAG TTGGGCCGTA ACTTCCAGGT GATCTACAAG 1980
CAGGCCAACG GCGACGGGCA GAAGGTGCAG GCTGCCGGCA ACAACATCGC GCAAACCGAC 2040
AGCGCCGTCG GCTCCAGCTG GGCCTAAAAC TGAACTTCAG TCGCGGCAGC ACACCAACCA 2100
GCCGGTGTGC TGCTGTGTCC TGCAGTTAAC TAGCACTCGA CCGCTGAGGT AGCGATGGAT 2160
CAACAGAGTA CCGGCACCGA CATCACCGTC AAGGTCAACG GCTTCTGGAT GCTTCAGGCG 2220
CTACTGGAIG TCGCCCACGT TGCGCCTGAG TTACGTTACC GGCCGTACGT CTCCACCGAT 2280
TCCAATGACT TGCCAAACGA GCACCCGAGG ATGGCGGTCA TGCGCGAGCA GGGCATTGTC 2340
GTCAACGAIG CGGTCGAACG ACAGGTCGCT GCCCGGATGA AGGTGCTTGC CGCACCTGAT 2400
CTTGAAGTCG TCGCCCTGCT GTCACGCGGC AATTGCTGT ACGCGTTCAT AGAGCAGCAG 2460
AACAAGCGGC CGGGTTCGCG TGACATCCCT GACAATGAGT TCCGGGTGGT GTTGGCCCGG 2520
CGAGCCAGC ACTGGGTGTC GGCGGTACGG GTTGGCAATG ACATCACCGT CGATGACGTG 2580
ACGGTCTCGG ATAGCGCCTC GATCGCCGCA CTGGTAATGG ACGGTCTGGA GTCGATTCAC 2640
CACGCCGACC CAGCCGCGAT CAACGCGGTC AACGTGCCAA TGGAGGAGAT CTCGTGCCGA 2700
ATTCGGCACG AGGCACGAGG CGGTGTCGGT GACGACGGGA TCGATCACGA TCATCGACCG 2760
GCCGGGATCC TTGGCGATCT CGTTGAGCAC GACCCGGCCC CGCGGGAAGC TCTGCGACAT 2820
CCATGGGTTC TTCCCG 2836

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 900 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AACATGCTGC ACGGGGTGCG TGACGGGCTG GTTCGCGACG CCAACAACTA CGAGCAGCAA 60
GAGCAGGCCT CCCAGCAGAT CCTCAGCAGC TAACGTCAGC CGCTGCAGCA CAATACTTTT 120
ACAAGCGAAG GAGAACAGGT TCGATGACCA TCAACTATCA GTTCGGTGAC GTCGACGCTC 180

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ACGGCGCCAT GATCCGCGCT CAGGGCGGGT TGCTGGAGGC CGAACATCAG GCCATCATTC    240
GTGATGTGTT GACCGCGAAT GACTTTTGGG GCGGCGCCGG TTCGGCGCAC TGCCAGGGGT    300
TCATTACCCA ATTGGCCCGT AACTTCCAGG TGATCTACGA ACAGGCCAAC GCCCACGGGC    360
AGAAGGTGCA GGCTGCCGGC AACAACATGG CGCAAACCGA CAGCGCCGTC GGCTCCAGCT    420
GGGCGTTACA CCAGGCCAAG GCCAGGGACG TGTTCTTACGA GTGAAGGTTC CTCGCGTGAT    480
CCTTCGGGTG GCAGTCTAGG TGGTCAGTAC TGGGGTGTTG GTGTTTTGCT GCTTGGCGGG    540
TTCTTCGGTG GTGGTCAGTG CTGCTCCGGC TCGGGTGAGG ACCTCGAGGC CCAGGTAGCG    600
CCGTCCTTCA ATCCATTCGT CGTGTTGTTC GGCGAGGACG GCTCCGACGA GGCGGATGAT    660
CGAGGCGCGG TCTGGGAAGA TCCCCACGAC GTCGGTTCGG CGTCGTACCT CTCGTTTGAG    720
GCGTTCTTGG GGGTTGATGG ACCAGATTTG GCGCCAGATC TTCTTGGGGA AGGCGGTGAA    780
CGCCAGCAGG TCGGTGCGGG CGGTGTTGAN GTGCTCGGCC ACCGGGGGGA GTTTGTCGGT    840
CAGAGCGTCG AGTACCCGAT CATATTGGGC AACAACTGAT TCGGCGTTGG GCTGGTCGTA    900
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1905 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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GCTCGCCGGA TGTGGGCGTC CGCGCAAAAC ATTTCCGGTG CGGGCTGGAG TGGCATGGCC     60
GAGGCGACCT GGCYAGACAC CATGGCCCAG ATGAATCAGG GTTTCGCAA CATCGTGAAC     120
ATGCTGCACG GGGTGCGTGA CGGGCTGGTT CGCGACGCCA ACAACTACGA GCAGCAAGAG     180
CAGGCYTCCC AGCAGATCCT CGCCAGCTAA CGTCAGCCGC TGCAGCACAA TACTTTTACA     240
AGCGAAGGAG AACAGGTTCG ATGACCATCA ACTATCAGTT CGGTGATGTC GACGCTCAGG     300
GCGCCATGAT CCCGCTCCAG GCCGGGTTGC TGGAGGCCGA CCATCAGGCC ATCATTCGTG     360
ATGTTGTGAC CGCGAGTGAC TTTTGGGGCG GCGCCGGTTC GGCGGCCTAC CAGGGGTTCA     420
TTACCCAGTT GGCCCGTAAC TTCCAGGTGA TCTACGAACA AGCCAACACC CACGGGCAGA     480
AGGTGCAAGG TGCCGGCAAC AACATGGCGC AAACCGACAG CGCCGTCGGC TCCAGCTGGG     540
CGTGACACCA GGCCAAGGCC AGGGACGTGG TGTACGAGTG AAGGTTCCTC GCGTGATCCT     600
TCGGGTGGCA GTCTAGGTGG TCAGTGCTGG GGTGTTGGTG GTTTGCTGCT TGGCGGGTTC     660
TTCGGTGGTG GTCAGTGCTG CTCCGGCTCG GGTGAGGACC TCGAGGCCCA GGTAGCGCCG     720
TCCTTCGATC CATTCGTCGT GTTGTTCGGC GAGGACGGCT CCGACGAGGC GGATGATCGA     780
GGCGCGGTCT GGGAAGATGC CCACGACGTC GGTTCGGCGT CGTACCTCTC GTTTGAGGCG     840
TTCTTGGGGG CCACCGCTTG GCGCCNAGGC ACTCCACGCC AATTCGTCNG ACGTAACAGC     900
GGTGGCCAAC GACTATGACT ACGACACCGT TTTTGCCAGG GCCCTCNAAA GGATCTGCGC     960
GTCCCGGCGA CAGGCTTTTT GGGATAAGTA CCTCCCGGCA TTCTATGAGT GTACTGCGGN    1020
CCGCGAAAAC CGCAAGGGAG TTGGGTGTGA CGGTTATTTC AAATGACGGG CGAATCNGGC    1080
GGCCAGCTGG CAGAATTCGC AGATTTCTTG ATCAACGTCC CGTCACGCGA CACCGGGCGA    1140
ATCCAGCAAT CTCACATCGT TTTTATTCAT GCCATCTCCG AACATGTCGA ACAGCGCCTT    1200
TTCGCCGCTG GCCRATAGGA AAGCCGATCC TTACGCGGCC ATTCAAAAGA TGGTCGCGGA    1260
ACGTGCGGGA CACCAATGGT GTCTCTTCCT CGATAGAGAC GGTGTCATCA ATCGACAAGT    1320
GGTCGGCGAC TACGTACGAA ACTGGCGGCA GTTTGAATGG TTGCCCGGKG CGGCGCGCGC    1380
GTTGAAGAAG CTCGGTGCAT GGGCTCCGTA CATCGTTGTC GTGACAAACC AGCAGGGCGT    1440
GGGTCCCGGA TTGATGAGCG CCGTCGACGT GATGGTGATA CATCGGCACC TCCAAATGCA    1500
GCTTGCATCC GATGGCGTGC TGATAGATGG ATTTCAGGTT TGCCCGCACC ACCGTTCGCA    1560
GCGGTGTGCC TGCCGTAAGC CGAGACCGGG TCTGGTCCTC GACTGGCTCG GACGACACCC    1620
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CGACAGTGAG CCATTGCTGA GCATCGTGGT TGCGGACAGC CTCAGCGATC TTGACATTGG    1680
CACACAACGT CGCCGCTGGT GCCGGTGCAT GTGCCAGTGT CCAGATAGGG GCGCCAGTT    1740
CTGGCGGTGT CGCTGACGCG TCATTTGACT CGCTCTGCGA GTTCGCTGTC GCAGTCGGAC    1800
ATGCGCGGGG GGAGCCGGAC TAATGGCGAT CTTGCGCGGG CGAGCGCGGT NGCGGNTCGG    1860
ACTNNCCGGT GGCGGGACAG ACGTGGAACC GTACTCGAGC CAGTT                    1905

(2) INFORMATION FOR SEQ ID NO:7:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 2521 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: double
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (vi) ORIGINAL SOURCE:
          (A) ORGANISM: Mycobacterium tuberculosis

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGGGATGCCG TGGTGGTTGG TATTGCGCCA ACCCTGGCGC TTGTCCCCCG GGTATCCAGG      60
TCCGGGTCGA CCATCAGCGC TGGACTGTTT CTCGGACTCG ACCGTGAACT GGCCGCCGCA     120
TTCGGATTCC TGCTGGCCAT TCCAGCTGTG TTCGCCTCCG GGTTGTTCTC GTTGCCCGAC     180
GCATTCGCAC CGGTAACCGA GGGCATGAGC GCTACTGCCC CGCAGTTGCT GGTGGCCACC     240
CTGATCGCGT TCGTCCTCGG TCTGACCGCG GTGGCCTGAC TGCTCGCGTT TCTGGTGCGA     300
CACACATGT ACTGGTTCGT CGGCTACCGG GTGCTCGTCG GGACGGGCAT GCTCGTGCTG     360
CTGGCTACCG GGACGGTAGC CGCGACATGA CCGTCATCTT GCTACGCCAT GCCLGTTCCA     420
CCTCGAACAC CGCGGGCGTG CTGGCCGGCC GGTCCGGCGT CGACCTCGAC GAGAAGGGGC     480
GCGAGCAGGC CACCGGGGTTG ATCGATCGAA TTRGTGACCT GCCGATCCGG GCGGTCGCGT     540
CTTCTCCAAT GCTCCGGTGT CAAGCACCG TCAAADCGCT GGCCGAGGCG CTGTGCCTTG     600
AGCCGCTCAT CGATGACCGG TTCTCCTGAG TCGACTACGG CGAATGGACT GGCAGAAAAA     660
TCGGTGACCT GGTCGACGAG CCGTTGTGGC GGGTAGTCGA GNCCCACCCC AGCGCCGCGG     720
TGTTTCCCGG CGGTGAGGGT TTGGCGCAGG TGGAGACGTG GTTGTCCTGA CGGATTTCCA     780
TGCCGGGGAA CACCAAGACG GGATCGGCAC TYGCGGTCGC CTGCGAAAAC CCGGCCGCCA     840
ATAGGGCGAC CGTCGCTGCG AATGCGCGTG GTACCAGGCG GACCACCTTG AACTCCCATC     900
CGTCGGCGCC AAGCGCATCG CCGCCGCCG GTTACGGCTA AGGCGTACCA AAACCCGACC     960
GTAATACTTC GGCAATGTCG GGTCNCGACG TTACCGAGAC GTGACCAGNG AGGCNGCGGC    1020
ATTGGATTTA TCGATGGTGC GCGGTTGCCA NCCCGGCGGT CCGAANAGGT AGCCCAGCCG    1080
ATCCCCGAGA CGTGTTGCCG ACCGCCACTC ACCGACGATC GCCACGTACT CGCGGGTCTG    1140
CAGCTTCCAG ATGTTGAACG TGTCGACCCG CTTGGTCGAG CCATAATGCG GTCGSAATAG    1200
CTCCGGCTGA AAGCTACCGA ACAGGCGGTC CCAGATGATG AGGATGCCCC CATAGTTCTT    1260
GTCCANATAC ACCGGGTCCA TTCCGTGGTG GACCCCGGTGG TGCGACGGCG TATTGAAGAC    1320
GAATTCGAAC CACCGCCGCA GCCTGTCGAT CCGTCGGTG TGSACCCAGA ACTNGTAGAT    1380
CAAGTTCAGC GACCAATTGC AGAACACCAT CCAAGGCGGA AGCCCCATCA GTGGCAGCGG    1440
AACCCACATG AGAATCTCGC CGCTGTTGTT CCANTTTCTG CCGCAGCCCG GTGCCGAAGT    1500
TGAAGTATTC GCTGGAGTGA TACGCCTGGT GGGTAGCCCA GATCAGCCGA ACTCGGTGGG    1560
CGATGCCGTG ATAGGAGTAG TACAGCAGAT CGACACCAAC GATCGCGATC ACTCCAGGTG    1620
ACCACCTGTG GCTGGCACGC TGCCAGGGGG CAANGTAGGC ATAGATTYGG GCATAACCGA    1680
GCAGGGCAAG GCACTTCCAG CCGGCGGTGG TGGCTATCGA AACCAGCCCC ATCGAGATGC    1740
TGGCCACCGA GTCGCGGGTG AGGTAAGCGC CCGAGGCGGG CCGTGGCTGC CCGGTAGCAG    1800
GGGTCTCGAT GCTTTCGAGC TYGCGGGCGG CCGTCCATTC GAGAATCAGC AGCAATAGAA    1860
AACATGGAAT GGCGAACAGT ACCGGGTCCC GCATTTCCTC GGGCAGCGCT GAGAAGAATC    1920
CGGCGACGGC ATGGCCGAGG CGACCTCGNT AGACACCATG ACCCAGATGA ATCAGGGTTT    1980
TCGCAACATC GTGAACATGC TGCACGGGGT GCGTGATCGG CTGGTTGCCG ACGCCAACAA    2040
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NTACGAACRG CAAGAAGAGG CGTCCGAGCA GATGCTCAGC AGCTGACCGG GCCCGACGAC      2100
TCAGGAGGRC ACATGACCAT CAACTATCAA TTCGGGAACG TCGACGCTCA CGGCGCCATG      2160
ATCGCGCGTC AGGCCGGGTC GCTGGAGGCC GAGCATCAGG CCATCATTTC TGATCTGTTG      2220
ACCGCGAGTG ACTTTTGGGG CGGCGCCGGT TCGGCGGCCT GCCAGGGGTT CATTACCCAG      2280
CTGGGCGTTA ACTTCCAGGT GATNTAGGAG CAGGCCAAGG CCCACGGGCA GAAGCTCGAG      2340
GCTGCTGGCA ACAACATGGC ACARACGGAC AGCGCCGTCG GCTCTAGCTG GGCATAAAGN      2400
TGGCTTAAGG CCCGCCCCGT CAATTACAAC GTGGCCGCAC ACCGGTTGRT GTGTGGCCAC      2460
GTTGTTATCT GAACGACTRA CTACTTCGAC GTGCTAAAGT CGGCGCGTTG ATCCCCGGTC      2520
NGATNGTGCT GAACTGGGAA GATGGCCTCA ATACCCTTGT TGNTGAAGGG ATTGAGGCTR      2580
TCGTGTTTCG TACTTTAGGC GATCAGTGCT GGTTGTCGGA GTCGCTGCTG CCCGACGAGG      2640
TGCGCCGACT GCCCGAGGAA GTGGCTCGGG TGGACGCATT GTTGGACGAT CCGGCGTTCT      2700
TCGCCCCGTT CGTGCCGTTC TTCGACCCGC GCAGGGGCCG GCCGTCGACG CGGATGGAGG      2760
TCTATCTCGA ATTGATGTTT GTGAAGTTCC GCTACCGGCT GGGCTATGAG TCGCTGTGCC      2820
GGGAGSTTGC TGATTCGATC ACCTGACGGC GGTTTTGCCG CATTGCGCTG GACGGTTCGG      2880
TGCCGCATTC GACCACATTG ATGAAGCTCA CCACGCGTTG C                          2921
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1704 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
CGCGATGGTC GTCAACGANG TCGACCGTCA CCACGGACTG ATCAACAAGT TCGCAGGCGA       60
CGCCGCCCTG GCCATCTTCG GAGCCCCGAA CCGCCTCCAC CGTCCCGAAG ACCCCGCGCT      120
GGCCGCCGCC CGGGCCATAN CCGANCGGCT GGCCNACGAG ATGCCCGAGG TCCAAGCCGG      180
CATCGGGGTT GCGGCCAGGC ANATCGTCGC CGGCAATGTC GGNGCCAAGC AAAGATTCNA      240
ATACACAGTG GTCGGCAAGC CGGTCAACCA NGCGGCCGCA TTGTGCNAAC TGGCCAAATC      300
ACACCCNGCG CGATTGGGTC TGGCCGGCTC GNCTCATGGT CACCCATTCC AAGGACTACT      360
TTGGCGTGGC GCAGGACCTG CGAAGTGGG CGAGTGAAAG CGCCAAAGCC GCCGGTGAGG      420
CCGCCAAGGC GTTGCCGGCC GCGGTTCCGG CCATTCCGAG TGCTGGCCTG AGCGGCGTTG      480
CNGGGCGCCT CGGTCAGGCG GCGTCGTCTC GGGGATTGAA GCTTCCGGCC GTTTGAACCG      540
CCACGACCCC GGCGGCGAGC CCCGCGGTGC TGGCCGCCTC CAACGGCCTC GGAGCCGCGG      600
CCGCGGCTGA AGTTCGACA CACGCGTTTG GCGGGATGCC GCTCATGTGT ANCGGTGCCG      660
GACGTCCGTT TAACAACTTC GCTGCCCCTC GATACGGATT CAAGCCGACC GTGATCGCCC      720
AACGCCCGGC TGGCGGATGA CCAACTACGT TCGTTGATCG AGGATCGAAT TCNACGATTC      780
AAAGGNAGGA ATTCATATGA CCTCNCGTTT TATGACGGAT CCGCACGCNA TNCGGGACAT      840
GGCGGGCCGT TTTGAGGTGC ACGCCCAGAC GGTGGAGGAC GAGGCTNGCN GGATGTGGGC      900
GTCCGCGCAA AACATTTCCG GTGCGGCCTG GAGTGGCATG GCCGAGGCGA CCTCGNTAGA      960
CACCATGGCC CNGATGAATC AGGCGTTTCN CAACATCGTG AACATYCTYL ACGGGGTNNG     1020
TGACGGGCTG GTTCGCGACG CCAAGAACTA CGAACAGCAA GNACAAGCCT CCCAGCAGAT     1080
CCTCAGCAGC TGACCCGGCC CGACGACTCA GGAGGACACA TGACCATCAA CTATCAATTC     1140
GGGGACGTCG ACGCTCATGG CGCCATGATC CGTGCTNTGG CCGGCTTTCT GGAGKCCCAG     1200
CATCAGGCCA TCATTTCTGA TGTGTTGAGC GCGAGTCACT TTTCGGGCGG CGCCGGTTCG     1260
GCGKCGTTGC AGKGGTTCAT TACCCAGTTG GGCCGTAACT TCCAGGTGAT TTACGAGCAG     1320
GCCAACGCCC ACGGGCAGAA GGTGCAGGCT GCGGGCAACA ACAGYGCCAC AACCGACAGC     1380
GCCGTNGGNT CCAGCTGGBC CTAACCCGGG TCNTAAGTTG GGTCCGCGCA GGGCGGGCCG     1440
```

```
ATCAGCGTNG ACTTTGGCGC CGGATACACG GGCATNTTNT NGTCGGGAAC ACTGTGNCCG   1500
CGTCAGNTGC CGGCTTCCCC TTGTTNGGCG ACGTGCTCGG TNATGGCTTT GAGCAGCGGT   1560
TCGCCGGCGC GGCCAATCAA TTGGTGCGGC TTGCCTNTAG CCCATTCGTG CGACGCCCGC   1620
GGCGCCGGCA GTTGTCCCTT GAAATAAGGA ATCACAGCAC GGGCGAACAG CTCATAGGAG   1680
TGAAAGGTTG CCGTGACGGG GCCC                                          1704
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2284 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
CCGTCCTTGC GTCTGGGCGC ATTGTGATCT GGGCCANTTG CCCCTCCACC CAGACCGCCC     60
CCAGCTTGTC GATCCAGCCG GCGACCCGGA TTGCCACCGC GCGAACCGGG AACGATTCT     120
CCGCTGAATT CTAGGTCACT TCGCAGTCGC GCGGGTGATC CTGTTGGCGA NCAGCGTCTG    180
GAACYGGCGT CNAACAGTTG CCGTAAGCCC AGCGTGTACG CGGTCAGCCC GACGCCGATG    240
CCGAATGCCT TGCCGNCCAA GCTGAGCCGC GCGGGCTCCA CCAAGAGCGT CACGGTGAGC    300
CAGCCAACCA GATGCAAGGC GACGATCACC GYAAAGTGCC GAATTCGGCA CGAGAGGTGC    360
TGGAAATCCA GCAATACGCC GGCGAGCCGA TCTCGTTYGA CCAGACCATC GGCGACGANG    420
GCGACAGNCA GTTTGGCGAT TTCATCGAAA ACAGCGAGGC GGTGGTGGNC GTCGACGCGG    480
TGTCCTTCAC TTTGCTGCAT GATCAACTNC ANTCGGTGCT GGACACGCTC TCCGAGCGTG    540
AGCCGGGCGT GGTGGGCTA CGCTTCGGCC TTACCGACGG CCAGCCGCGC ACCCTTGACG    600
AGATCGGCCA GGTCTACGGC GTGACCCGGG AACGCATCCG CCAGATCGAA TCCAAGACTA    660
TGTCGAAGTT GCGCCATCCG AGCCGCTCAC AGGTCCTGCG CGACTATCGT GCCGAATTCG    720
GCACGAGCCG TTTTGAGGTG CACGCCCAGA CGGTCGAGGA CGAGGCTCGC CGGATGTGGC    780
CGTCCCGCCA AAACATTTCC GGTGCGGGCT GGAGTGGCAT GCCGAANGCG ACCTCGCTAG    840
ACACCATGGC CCAGATGAAT CAGCGTTTC GCAACATCGT GAACATGCTG CACTGGGTGC    900
GTGACGGGCT GGTTGGCGAC GCCAACAACT ACGAACAGCA AGAGCAGGCC TCCCAGCAGA    960
TCCTCAGCAG CTGACCCGGC CCGACGACTC AGGAGGACAC ATGACCATCA ACTATCAATT   1020
CGGGGACGTC GACGCTCATG GCGCCATGAT CGGCGCTCTG GCCGGGTTGC TGGAGCCGTA   1080
GCATCAGGCC ATCATTTCTC AKTTGTTGAC CGCGATGTAC TTTTGGGCGC GCCCCGGTTC   1140
GGCGGCCTGC CAGGGGTTCA TCACCCAGTT GGGCGGTAAC TTCCAGGTGA TCTACGAGCA   1200
GGCCAACGGC GACGGGCAGA AGGTGCAGGC TGCCGGCAAC AACATGGCAC AAACCGACAG   1260
CGCCGTCGGC TCCAGCTGGG CCTAACCUGG GTCCTAAGTT GGGTCGGCGC AGGGCGGGCC   1320
GATCAGCGTC GACTTTGGCG CCGGATACAC GGGCATGTNG TNGTCGGGAA CACTGCGCCC   1380
GCGTCAGCTG CCCGCTTCCC CTTGTTGGGC GACGTGCTCG GTNATGGCTT TGAGCAGCGG   1440
TTCGCCGGCG CGGCCAATCA ATTGGTGCGG CTTGCCTCTA GCCTCGTGCC GAATTCGGCA   1500
CGAGGGTGGT GGTGCCGCGC TATCGGCAGC ACGTGAGCTC CACGACGAAC TCATCCCAGT   1560
GCTGGGTTCC GCGAAGTTCG GCATCGAGGT GTCGGCCGGA AGGGCCATCG CCGGCCACAT   1620
CGGCGCTCAA GCCGCGTTCG AGTACACGGT CATTGCCGAC CCGGTCAACG AGGCCGCCCG   1680
GCTCACCGAA CTGGCCAAAG TCGAGGATGG CCACGTTCTG GCGTCGGCGA TCGCGGTCAG   1740
TGGCGCCCTG GACGCCGAAG CATTGTGTTG GGATGTTGGC GAGGTGGTTG AGCTCCGGTC   1800
ACGTGCTGCA CGCACCCAAC TAGCCAGGCC AATGAATNTG GCNGCAGCCG AAGAGGTTTC   1860
CAGCGAAGTA CGCGGCTAGT CGCGCTTGGC TGCNTTCTTC GCCGGCACCT TCCGGNCAGC   1920
TTTCCTGGCT GCCCGTTTTG CCGGACCCCG GGCTCGGGCA TCGGCCAACA GCTTNNCGGC   1980
GCGCTGGTCG GTPATGGAAG CCACGTNGTC GCCCTTACGC AGGCTGGCAT TGGTCTCACC   2040
```

```
GTCGGTGACG TACGGCCCGA ATCGGTCGTC CTTGATNACC ATTGGCTTGC CAGACGCCGC     2100
ATNTGNTCGC AGCTCGCGCA GCGGCGGAGC CGAAGCGCTT TGCCGGCCAC GACNTTTCGC     2160
CTCTGNGTAG ATNTTCAGGG CTTCGTCGAG CGNGATGGTG AATATATGGT CTTCGGTGAC     2220
CAGTGATCGA GAATCGTTGC CGCGCTTTAG ATACGGTCNT TAGCGCCCGT TCTGCGCGGT     2280
GATNTC                                                                2286

(2) INFORMATION FOR SEQ ID NO:10:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 1136 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: double
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (vi) ORIGINAL SOURCE:
          (A) ORGANISM: Mycobacterium tuberculosis

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGCATCTTC CCCGACCGCG CCTCGATCAT CGGCTCGTTC GGTGCCGTCC TCGCCGAACA       60
ACACGACGAA TCGATCGAAG GATTGCGCTA CCTCGGCCTC GAGGTCCTCA CCCGAGCCCG      120
AGCAGCACTG ACCAGGCACG AAGAACCTCC AAGCAGCAAA CCACCAACAC CCCAGCACTG      180
ACCACCTAGA CTGCCACCCG AAGGATCACG CGAGCAACCT TCACTCGTAC ACCACGTCCC      240
TGGCCTTGGC CTGGTGTCAG GCCCAGCTGG AGCCAACCGC GCTGTCGGTT TGCGCCATGT      300
TGTTGCCGGC AGCCTGCACC TTCTGCCCGT GGGCGTTGGC CTGCTCGTAG ATCACCTGGA      360
AGTTACGGCC CAACTGGGTA ATGAACCCCT GGCAGGCCGC CGAACCGGCG CCGCCCCAAA      420
AGTCACTCGC GGTCAACACA TCACGAATGA TGGCGTGTGT CTCGGCCTCC AGCAACCCGG      480
CCTGAGCGCG GATCATGGCG CCGTGAGCGT CGACATCACC GAACTGATAG TTGATGGTCA      540
TCGAACCTGT TCTCCTTCGC TTGTAAAAGT ATTGTGCTGC AGCGGCTGAC GTTAGCTGCT      600
GAGGATCTGC TGGGAGGCCT GCTCTTGCCT CGTGCCGAAT TCGGCACGAG AGGCTGCCTT      660
CGAAGAAATC CTTTGAGAAT TCGCCAAGGC CGTCGACCCA GCATCGGGCC AGCTCGCCAG      720
CCGCGCCGGC TGGCAACCGT TCCCGCTCGA GAAAGACCTG GAGGAATACC ATTGACGAAC      780
GACCTCCCAG ACGTCCGAGA GCGTGACGGC GGTCCACGTC CCGCTCCTCC TGCTGGCGGG      840
CCACGGTTGT CAGACGTGTG GGTTTACAAC GGGCGGGCGT ACGACCTGAG TGAGTGGATT      900
TCCAAGCATC CCGCCGGCGC CTTNTTCATT GGGCGGACCA AGAACCGCGA CATCACCGCA      960
ATCGTCAAGT CCTACCATCG TGATCCGGCG ATTGTCGAGC GAATCGTGCA GCGGAGGTAC     1020
GCGTTGGGCC GCGACGCAAC CCCTAGGGAC ATCCACCCCA AGCACAATGC ACCGGCATTT     1080
CTGTTCAAAG ACGACTTCAA CAGCTGGCGG GACACCCCGA AGFATCGATT NGACCA         1136

(2) INFORMATION FOR SEQ ID NO:11:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 967 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: double
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (vi) ORIGINAL SOURCE:
          (A) ORGANISM: Mycobacterium tuberculosis

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
```

TGAGCGCCAA CCCTACCGTC GGTTTGTCAC ACGGACCGCA TCGCCTGCTC CGCGGACTGC 60
CGCTAGGGTC GCGGATCACT CGGCGTAGCG GCGCCTTTTC CCACCGATAT GGGTTCCGTC 120
ACAGTGTTGT TGCCCGCCCG CCATTCGGCC GATAACGCCA TGACCTCAGC TCGGCAGAAA 180
TGACAATGCT CCCAAAGGCG TGAGCACCCG AAGACAACTA AGCAGCAGAT CGCATGCCGT 240
TTGTGACTAC CCAACCAGAA GCACTGGCGG CGGCGGCCGG CAGTCTGCAG GGAATCGGTT 300
CGGCATTGAA CGCCGAGAAT GCGGCTGCGG CGACTCCCAC GACGGGGTTG GTCCGGCGGC 360
CGCCGATGAA NTGTCGGCGC TGACGGCGGC TCAGTTCGCG GCACACGCCC AGATCTATCA 420
GGCCGTCGGC GCCCAGGCGT CGTCGATTCA CGAGATGTTC GTCAACTCTC TACAGATGAG 480
CTCAGGGTCG TATGCTGCTA CCGAGGCCGC CAACGCGGCC GCGGCCGGNT AGAGGAGTCA 540
CTGCGATGGA TTTTGGGGCG TTGCCGCCGG AGATCAATTC GGTGCGGATG IATGCCGTTC 600
CTGGCTCGGC ACCAATGGTC GCTGCGGCGT CGGCCTGGAA CGGGTTGGCC GCGGAGCTGA 660
GTTCGGCCGC CACTGGTTAT GAGAGGGTGA TCACTCAACT CAGCAGTGAG GGGTGGCTAG 720
GTCCGCCGTC AGCGGCGATG GCCGAGGCAG TTGCGCCGTA TGTGGCGTGG ATGAGTGCCG 780
CTGCGGCGCA AGCCGAGCAG GCGGCCACAC AGGCCAGGGC CGCCGCGGCC GCTTTTGAGG 840
CGGCGTTTGC CGCGACGGTG CTCCGCCGST TGATCGCGGC CAACCGGGCT TCGTTGATGC 900
AGCTGATCTC GACGAATGTC TTTGCTCAGA ACACCTCGGC GATCGCGGCC GCCGAAGCTC 960
AGTACGG 967

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 585 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGGATTCCGA TAGCGGTTTC GGCCCCTCGA CGGGCGACCA CGGCGCGCAG GCCTCCGAAC 60
GGGGGGCCGG GACGCTGGGA TTCGCCGGGA CCGCAACCAA ACAACTGCCG GTCCGGGCGG 120
TCGGGCTGAC CGCACTGGCC GGTGATGAGT TCGGCAACGG CGCCCCGATG CCGATGGTGC 180
CGGGAACGTG GGAGCAGGGC AGCAAGGAGC CCGAGGCGCC CGACGGATCG GGAAGAGGGG 240
GAGGCGACGG CTTACCGCAC GACAGCAAGT AACCGAATTC CGAATCACGT GGACCCGCAC 300
GGGTCGAAAG GAGAGATTTT ATCAGCCTTT TGGATTCTCA TATCCCACAG TTGGTGGCCT 360
CCCAGTCGAC GTTTGCCGCC AAGGCGGGGC TGATGCGGCA CACGATCGGT CAGGCCGAGC 420
AGGCGGCGAT GTCGGCTCAG GCGTTTCACC AGGGGAGTC GTCGGCGGCG TTTCAGCCGG 480
CCCATGCCCG GTTTGTGGCG GCGGAAYGCA AAGTCAACAC CTTGTTGGAT GTCGCGCAGG 540
GGAATCTGGG TGARGCCGCC GGTACCTATG TGGCCGCCAA TGCTG 585

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 148 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Leu Val Thr Thr Asn Phe Phe Gly Val Asn Thr Ile Pro Ile Ala
1 5 10 15
Leu Asn Glu Ala Asp Tyr Leu Arg Met Trp Ile Gln Ala Ala Thr Val
20 25 30
Met Ser Met Tyr Gln Ala Val Xaa His Glu Ile Trp Cys Leu His Glu
35 40 45
Xaa Ala Ser Ser Gly Lys Pro Trp Ala Ser Ile Thr Thr Gly Ala Pro
50 55 60
Gly Ser Pro Ala Ser Thr Thr Arg Ser Arg Thr Pro Leu Val Ser Thr
65 70 75 80
Asn Arg Xaa Val Xaa Ala Pro Ile Val Ser Pro Asn His Thr Gly His
85 90 95
Arg Pro Glu Lys Gly Leu Gly Ser Xaa Gln Arg Arg Leu Ser Arg Val
100 105 110
Leu Pro Arg Ile Ile Asp Arg Pro Ala Gly Pro Xaa Gly Pro Pro Leu
115 120 125
Thr Ser Gly Ser His Phe Leu Cys Ser Trp His Gly Tyr Ser Ser Gln
130 135 140

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 352 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

His Ala Leu Ala Ala Gln Tyr Thr Glu Ile Ala Thr Gln Leu Ala Ser
1 5 10 15
Val Leu Ala Ala Val Gln Ala Ser Ser Trp Gln Gly Pro Ser Ala Asp
20 25 30
Arg Phe Val Val Ala His Gln Pro Phe Arg Tyr Trp Leu Thr His Ala
35 40 45
Ala Thr Val Ala Thr Ala Ala Ala Ala Ala His Xaa Thr Ala Ala Ala
50 55 60
Gly Tyr Thr Ser Ala Leu Gly Gly Met Pro Thr Leu Ala Glu Leu Ala
65 70 75 80
Ala Asn His Ala Met His Gly Ala Leu Val Thr Thr Asn Phe Phe Gly
85 90 95
Val Asn Thr Ile Pro Ile Ala Leu Asn Glu Ala Asp Tyr Leu Arg Met
100 105 110
Trp Ile Gln Ala Ala Thr Val Met Ser His Tyr Gln Ala Val Ala His
115 120 125

```
Glu Ser Val Ala Ala Thr Pro Ser Thr Pro Pro Ala Pro Gln Ile Val
    130                 135                 140
Thr Ser Ala Ala Ser Ser Ala Ala Ser Ser Ser Phe Pro Asp Pro Thr
145                 150                 155                 160
Lys Leu Ile Leu Gln Leu Leu Lys Asp Phe Leu Glu Leu Leu Arg Tyr
                165                 170                 175
Leu Ala Val Glu Leu Leu Pro Gly Pro Leu Gly Asp Leu Ile Ala Gln
            180                 185                 190
Val Leu Asp Trp Phe Ile Ser Phe Val Ser Gly Pro Val Phe Thr Phe
        195                 200                 205
Leu Ala Tyr Leu Val Leu Asp Pro Leu Ile Tyr Phe Gly Pro Phe Ala
    210                 215                 220
Pro Leu Thr Ser Pro Val Leu Leu Pro Ala Val Glu Leu Arg Asn Arg
225                 230                 235                 240
Leu Lys Thr Ala Thr Gly Leu Thr Leu Pro Pro Thr Val Ile Phe Asp
                245                 250                 255
His Pro Thr Pro Thr Ala Val Ala Glu Tyr Val Ala Glu Gln Met Ser
            260                 265                 270
Gly Ser Arg Pro Thr Glu Ser Gly Asp Pro Thr Ser Glu Val Val Glu
        275                 280                 285
Pro Ala Arg Ala Glu Phe Gly Thr Ser Ala Val His Gln Ile Pro Pro
    290                 295                 300
Arg Pro Ala Asp Thr Arg Arg Ala Cys Arg His Arg Asp Asp Val Pro
305                 310                 315                 320
Arg Asp Ser Arg Ile Ala Gln His Arg Asp Gly Ala Gly Leu Asp Pro
                325                 330                 335
Thr Glu Arg Gly Thr Ser Glu Gly Asp Gln Gly Leu Val Ser Gly Trp
            340                 345                 350
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Met Asp Phe Gly Ala Leu Pro Pro Glu Val Asn Ser Val Arg Met Tyr
1               5                   10                  15
Ala Val Pro Gly Ser Ala Pro Met Val Ala Ala Ala Ser Ala Trp Asn
            20                  25                  30
Gly Leu Ala Ala Glu Leu Ser Ser Ala Ala Thr Gly Tyr Glu Thr Val
        35                  40                  45
Ile Thr Gln Leu Ser Ser Glu Gly Trp Leu Gly Pro Ala Ser Ala Ala
    50                  55                  60
Met Ala Glu Ala Val Ala Pro Tyr Val Ala Trp Met Ser Ala Ala Ala
65                  70                  75                  80
Ala Gln Ala Glu Gln Ala Ala Thr Gln Ala Arg Ala Ala Ala Ala Ala
```

```
                85                  90                  95
Phe Glu Ala Ala Phe Ala Ala Thr Val Pro Pro Pro Leu Ile Ala Ala
            100                 105                 110
Asn Arg Ala Ser Leu Met Gln Leu Ile Ser Thr Asn Val Phe Gly Gln
        115                 120                 125
Asn Thr Ser Ala Ile Ala Ala Ala Glu Ala Gln Tyr Gly
    130                 135                 140
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 58 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Met Ala Ser Arg Phe Met Thr Asp Pro His Ala Met Arg Asp Met Ala
1               5                  10                  15
Gly Arg Phe Glu Val His Ala Gln Thr Val Glu Asp Glu Ala Arg Arg
            20                  25                  30
Met Trp Ala Ser Ala Glu Asn Ile Ser Gly Ala Gly Trp Ser Gly Met
        35                  40                  45
Ala Glu Ala Thr Ser Leu Asp Thr Met Thr
    50                  55
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
1               5                  10                  15
Ile Arg Ala Gln Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val
            20                  25                  30
Arg Asp Val Leu Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val
        35                  40                  45
Ala Cys Gln Glu Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile
    50                  55                  60
```

Tyr Glu Gln
65

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 58 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Ala Ser Arg Phe Met Thr Asp Pro His Ala Met Arg Asp Met Ala
1 5 10 15
Gly Arg Phe Glu Val His Ala Gln Thr Val Glu Asp Glu Ala Arg Arg
20 25 30
Met Trp Ala Ser Ala Gln Asn Ile Ser Gly Ala Gly Trp Ser Gly Met
35 40 45
Ala Glu Ala Thr Ser Leu Asp Thr Met Thr
50 55

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
1 5 10 15
Ile Arg Ala Gln Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val
20 25 30
Arg Asp Val Leu Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val
35 40 45
Ala Cys Gln Glu Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile
50 55 60
Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn
65 70 75 80
Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser Trp Ala
85 90

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Asn Met Leu His Gly Val Arg Asp Gly Leu Val Arg Asp Ala Asn Asn
1 5 10 15
Tyr Glu Gln Gln Glu Gln Ala Ser Gln Gln Ile Leu Ser Ser
20 25 30

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
1 5 10 15
Ile Arg Ala Gln Ala Gly Leu Leu Glu Ala Glu His Gln Ala Ile Ile
20 25 30
Arg Asp Val Leu Thr Ala Ser Asp Phe Trp Gly Gly Ala Gly Ser Ala
35 40 45
Ala Cys Gln Gly Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile
50 55 60
Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn
65 70 75 80
Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser Trp Ala
85 90

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Ala Arg Arg Met Trp Ala Ser Ala Gln Asn Ile Ser Gly Ala Gly Trp
1               5                  10                  15
Ser Gly Met Ala Glu Ala Thr Ser Leu Asp Thr Met Ala Gln Met Asn
            20                  25                  30
Gln Ala Phe Arg Asn Ile Val Asn Met Leu His Gly Val Arg Asp Gly
        35                  40                  45
Leu Val Arg Asp Ala Asn Asn Tyr Glu Gln Glu Glu Gln Ala Ser Gln
    50                  55                  60
Gln Ile Leu Ser Ser
65
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
1               5                  10                  15
Ile Arg Ala Gln Ala Gly Leu Leu Glu Ala Gln His Gln Ala Ile Ile
            20                  25                  30
Arg Asp Val Leu Thr Ala Ser Asp Phe Trp Gly Gly Ala Gly Ser Ala
        35                  40                  45
Ala Cys Gln Gly Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile
    50                  55                  60
Tyr Glu Gln Ala Asn Thr His Gly Gln Lys Val Gln Ala Ala Gly Asn
65                  70                  75                  80
Asn Met Ala Gln Thr Asp Ser Ala Val Xaa Ser Ser Trp Ala
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Gly Met Ala Glu Ala Thr Ser Xaa Asp Thr Met Thr Gln Met Asn Gln
1               5                  10                  15
Ala Phe Arg Asn Ile Val Asn Met Leu His Gly Val Arg Asp Gly Leu
            20                  25                  30
Val Arg Asp Ala Asn Xaa Tyr Glu Gln Gln Glu Gln Ala Ser Gln Gln
        35                  40                  45
Ile Leu Ser Ser
    50
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
1               5                  10                  15
Ile Arg Ala Gln Ala Gly Ser Leu Glu Ala Glu His Gln Ala Ile Ile
            20                  25                  30
Ser Asp Val Leu Thr Ala Ser Asp Phe Trp Gly Gly Ala Gly Ser Ala
        35                  40                  45
Ala Cys Gln Gly Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Xaa
    50                  55                  60
Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn
65                  70                  75                  80
Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser Trp Ala
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Thr Ser Arg Phe Met Thr Asp Pro His Ala Met Arg Asp Met Ala
1               5                  10                  15
Gly Arg Phe Glu Val His Ala Gln Thr Val Glu Asp Glu Ala Arg Arg
            20                  25                  30
Met Trp Ala Ser Ala Gln Asn Ile Ser Gly Ala Gly Trp Ser Gly Met
        35                  40                  45
Ala Glu Ala Thr Ser Leu Asp Thr Met Ala Glu Met Asn Gln Ala Phe
    50                  55                  60
Arg Asn Ile Val Asn Met Leu His Gly Val Arg Asp Gly Leu Val Arg
65                  70                  75                  80
Asp Ala Asn Asn Tyr Glu Gln Gln Glu Gln Ala Ser Gln Gln Ile Leu
                85                  90                  95
Ser Ser
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
1               5                  10                  15
Ile Arg Ala Xaa Ala Gly Leu Leu Glu Ala Glu His Gln Ala Ile Ile
            20                  25                  30
Ser Asp Val Leu Thr Ala Ser Asp Phe Trp Gly Gly Ala Gly Ser Ala
        35                  40                  45
Ala Cys Gln Gly Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile
    50                  55                  60
Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn
65                  70                  75                  80
Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser Trp Ala
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Arg Phe Glu Val His Ala Gln Thr Val Glu Asp Glu Ala Arg Arg Met
1 5 10 15
Trp Ala Ser Ala Gln Asn Ile Ser Gly Ala Gly Trp Ser Gly Met Ala
20 25 30
Xaa Ala Thr Ser Leu Asp Thr Met Ala Gln Met Asn Gln Ala Phe Arg
35 40 45
Asn Ile Val Asn Met Leu His Gly Val Arg Asp Gly Leu Val Arg Asp
50 55 60
Ala Asn Asn Tyr Glu Gln Gln Glu Gln Ala Ser Glu Gln Ile Leu Ser
65 70 75 80
Ser

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Met Thr Ile Asn Tyr Glu Phe Gly Asp Val Asp Ala His Gly Ala Met
1 5 10 15
Ile Arg Ala Leu Ala Gly Leu Leu Glu Ala Glu His Gln Ala Ile Ile
20 25 30
Ser Asp Val Leu Thr Ala Ser Asp Phe Trp Gly Gly Ala Gly Ser Ala
35 40 45
Ala Cys Gln Gly Phe Ile Thr Glu Leu Gly Arg Asn Phe Gln Val Ile
50 55 60
Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn
65 70 75 80
Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser Trp Ala
85 90

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gln Glu Gln Ala Ser Gln Gln Ile Leu Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
1 5 10 15
Ile Arg Ala Gln Ala Gly Leu Leu Glu Ala Glu His Gln Ala Ile Ile
20 25 30
Arg Asp Val Leu Thr Ala Ser Asp Phe Trp Gly Gly Ala Gly Ser Ala
35 40 45
Ala Cys Gln Gly Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile
50 55 60
Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn
65 70 75 80
Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser Trp Ala
85 90

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met Ser Phe Val Thr Thr Gln Pro Glu Ala Leu Ala Ala Ala Ala Ala
1 5 10 15
Asn Leu Gln Gly Ile Gly Thr Thr Met Asn Ala Gln Asn Ala Ala Ala